REMARKS

Claims 1-15 are currently pending. Claims 14 and 15 were withdrawn by the Examiner. Claim 1 has been amended to recite "positive MR-imaging probes and recording MRI images of the cells by use of T1-weighted sequences." Support for the amendment to claim 1 can be found at page 3 line 24 and page 7 lines 1-4 of the specification. Therefore, no new matter has been added.

35 U.S.C. § 102

Klaveness

Claims 1, 2, 4-7 and 12 have been rejected under 35 U.S.C. § 102(b) as anticipated by U.S. Pat. No. 4.985,233 ("Klaveness") as evidenced by the Encyclopedia Britannica Article "Reticuloendothelial System" ("Britannica") because Klaveness allegedly discloses the claimed steps of exposing, internalizing and degrading.

In order for a reference to anticipate a claim, it must disclose each and every element of the claim, either explicitly or inherently. MPEP § 2131. Applicants respectfully traverse the § 102(b) rejection over Klaveness as evidenced by Britannica because Klaveness fails to disclose each and every claim limitation.

As amended, claim 1 recites:

> A method of cellular labeling comprising the steps of:
>
> (a) exposing insoluble particles comprising at least one paramagnetic complex of lanthanide or transition metal chelates to cells;
>
> (b) internalizing the insoluble particles inside the cells ~~and~~
>
> (c) degrading the insoluble particles by enzymes or by effectors in the environment surrounding the insoluble particles to form water soluble positive MR-imaging probes and
>
> (d) recording MRI images of the cells by use of T1-weighted sequences.

First, Klaveness fails to disclose that the administered insoluble particles must be first internalized in the targeted cells and then be degraded by enzymes or effectors inside the cells in order to be able to provide positive MRI-imaging probes and allow to record MRI images of the targeted cells by use of T1-weighted sequences. Applicants respectfully point out that without degradation of the particles, the release of water-soluble MRI-imaging probes is in no way possible and, as a consequence, a positive contrast cannot be generated. In other words, the degradation of the "relaxometrically silent" insoluble particles to "relaxometrically active" water-soluble MRI-imaging probes (page 4, line 6) is a necessary step for recording positive MRI imaging.

In contrast, Klaveness merely describes a method of diagnosis comprising administering a water-insoluble, water-swellable, hydroxyl-group-containing particulate macromolecular product which is cross-linked to form a three-dimensional network, and which carries within its cavities at least one non-radioactive paramagnetic metal species and which generates an NMR or ultrasound image of said region (Klaveness at claim 1). Thus, Klaveness fails to disclose a method of cellular labeling.

As correctly pointed out by the Examiner, Klaveness's contrast agents are degradable in the body to small, water-soluble excretable fragments (see column 5, lines 16-18) Thus, 'for example, degradable particles.... having such a size (...) that they are taken up by the reticuloendothelial system (RES) of e.g. the liver after parenteral administration are of special interest for investigating the liver (column 5, lines 45-50)."

However, Applicants points out that Klaveness' particles provide MRI imaging independently of their degradation. In fact, for example, insoluble particles which are not degradable in the body may be used for investigation of body cavities (column 5, lines 1214). Thus, in the method of Klaveness, degradation of the insoluble particles is not mandatory for obtaining water-soluble positive MRI-imaging probes (i.e. for recording MRI images of a concerned cell), but is only a way to reduce the size of the insoluble macromolecules and to form better excretable fragments.

Applicants note the Examiner's inherency argument that, upon introduction into the cells, the Klaveness particles would be inherently exposed to enzymes or effectors in the environment and would be degraded because Klaveness also describes that the particles are degradable (Office Action at p. 7).

Applicants respectfully disagree and point out that what the Britannica article actually describes is that the "Reticuloendothelial system... is a class of cells ... that take up particular substances" (page 1, first paragraph) and that "Reticuloendothelial cells are phagocityc" (page 1, second paragraph). This makes it clear that "to be taken up by RES system" means "to enter RES System cells through phagocytic activity" or "to be phagocytised by RES cells" (which is the same). Accordingly, the Encyclopaedia Britannica article confirms that the low dimension Klaveness' particles are internalized by RES cells through phagocytic activity. The Britannica article, however, is silent on the precise fate of the engulfed material and the only teaching in this respect is that the engulfed material is destroyed. More precisely, the article recites: "Reticuloendothelial cells are phagocytic; i.e. they can engulf and destroy bacteria, virus and other foreign substances" (page 1, second paragraph). Therefore, this article mentions only destruction

of phagocytised substances by RES System and does not describe any reaction performed by specific enzymes or effectors inside RES cells that would allow to form water-soluble MR-imaging probes, i.e. to release single units of paramagnetic chelates that would allow to record MRI images of the concerned cells by use of T1-weighted sequences. Thus, the Britannica article fails to show that the Klaveness particles would be inherently exposed to enzymes or effectors in the environment to form water-soluble MR-imaging probes.

Second, Klaveness fails to disclose a method of cellular labeling comprising first internalizing the insoluble particles inside the cells and then degrading the insoluble particles by enzymes or by effectors to form water-soluble positive MR-imaging probes. Instead, as explained at column 5, lines 10-50, Klaveness' degradable particles are firstly degraded inside the body to provide fragments of suitable low dimension and then the said fragments are taken up by the RES system thus, allowing liver imaging:

> Insoluble particles which are degradable in the body to smaller, water-soluble excretable fragments may be chosen, for example, for parenteral administration....The particles can be produced with the desired size and, if desired, can be provided with metal binding structures to which the paramagnetic metal species may be chemically bound. For example, degradable particles according to the invention having such a size (e.g. about 0.1-3 .mu.m, for instance 0.5-2 .mu.m in water swollen state) that they are taken up by the reticuloendothelial system (RES) of e.g. the liver after parenteral administration are of special interest, e.g. for investigations of the liver.

It stems from this that degradation occurs outside RES cells, i.e. before RES cell internalization.

Third, Klaveness fails to disclose a method of cellular labeling wherein the administered particles are insoluble due to hydrophobic substituents bound to the surface

of a chelating cage (see instant claim 2). In fact, Klaveness instead describes hydroxyl-group containing particulates (Klaveness at claim 1).

Finally, Klaveness also fails to disclose a method of cellular labeling wherein the particles are functionalised with synthons able to target the particles so as to interact with specific recognition sites on the outer membrane of the cells of interest, thus stimulating cell-internalization (instant claim 13 limitation).

Therefore, Klaveness fails to disclose each and every limitation of present claim 1, namely a method of cellular labeling comprising degrading the insoluble particles by enzymes or by effectors in the environment surrounding the insoluble particles, to form water-soluble positive MR-imaging probes. In view of the above, Applicants believe that Klaveness fails to anticipate the invention as presently claimed.

In conclusion, Klaveness and the Britannica article do not disclose a method of cellular labeling wherein an insoluble particulate macromolecular product is first exposed to targeted cells, then is recognized, internalized and finally degraded by enzymes or suitable effectors inside the cells giving rise to water-soluble MR-imaging probes for recording MRI-images of the concerned cells by use of T1-weighted sequences.

Shitaka

Claims 1, 4, 6 and 8-10 were rejected under 35 U.S.C. § 102(a) as anticipated by Shikata et al. (Eur. J.Pharmaceutics and Biopharmaceutics, 2002, 53, p. 57-63) ("Shikata") because, *inter alia*, Shikata discloses integrating Gd-NCT with MRI diagnosis and claimed steps of exposing, internalizing and degrading.

Applicants respectfully traverse the § 102(a) rejection because Shitaka fails to disclose each and every limitation of the claims.

Shikata fails to disclose the degradation of the insoluble particles by enzymes or by effectors in the surrounding environment to form water-soluble positive MR-imaging probes. Instead, Shikata's insoluble Gd-nanoCPs nanoparticles as such, once internalized inside the targeted tumor cells, provide the desired therapeutic effect, i.e. without requiring degradation (either spontaneous or enzymatic) in the surrounding environment, so as to form water-soluble positive MR-imaging probes.

In the Examiner's opinion, "upon introduction into the cells, the particles would inherently be exposed to enzymes or effectors in the environment to thereby degrade the particles, as Shikata also teaches that chitosan is biodegradable (bioerodible)". Thus, "Since Shikata performs all of the active claim steps recited, Shikata accomplishes the claimed method" (official action page 7, bottom).

Applicant disagrees and points out that the adjective "Biodegradable" or "bioerodible" does not define particles "able to form a water-soluble positive MR-imaging probe, allowing MRI image registration", but merely characterizes a compound that may be destroyed or degraded inside the body by suitable effectors.

Shikata also fails to disclose how to make an MRI diagnosis and furthermore, does not teach a method of cellular labeling comprising recording MRI images of the targeted cells by use of T1-weighted sequences. Shikata instead describes the accumulation of gadolinium loaded as gadopentaacetic acid (Gd-DTPA) in chitosan nanoparticles (Gd-nanoCPs) designed for gadolinium neutron-capture therapy (page 57, abstract and left column). As expressly reported in Shikata, Gadolinium neutron-capture

therapy (Gd-NCT) is a cancer therapy which utilizes 7-rays and electrons emitted by 157Gd (n, y) '58Gd reaction in order to kill tumor cells (page 57, left column). This therapeutic technique does not involve the recording of T1-weighted sequences and does not provide MRI images.

Accordingly, even if the cited reference does not exclude an inherent exposition of chitosan derivatives to enzymes or effectors promoting their degradation, it indeed fails to disclose degradation as a mandatory step for obtaining a water-soluble positive MR-imaging probe allowing to record MRI images of the concerned cell by use of T1-weighted sequences.

For these reasons, Shikata fails to anticipate the instant invention as presently claimed.

Kalbaka

Claims 1-3 and 6 were rejected under 35 U.S.C. § 102(a) as anticipated by Kabalka *et al.* (Mag. Res. In Medicine, 1988, 8, 53, p 89-95) ("Kabalka") as evidenced by the Britannica article because, *inter alia*, the article shows that a characteristic not explicitly disclosed in Kabalka (*i.e.* internalisation of the particles by RES cells) is inherent.

Applicants first point out that Kabalka fails to disclose the degradation of the insoluble particles by enzymes or by effectors in the surrounding environment to form water-soluble positive MR-imaging probes. Instead, Kabalka describes that the liposomes are so insoluble that they remain in the liver indefinitely, especially those which contain Gd-DTPA-SA (page 94, second paragraph).

More specifically, Kabalka describes liposomes including amphiphatic gadolinium complexes as part of the liposomal lamella (page 89, abstract and bottom of

the Introduction); these liposomes are said to exhibit excellent in vivo stability (they clear away from the liver over a period of days) and to provide 150% enhancement of the T 1 - signal in mouse liver (page 94) after i.v. administration (see abstract, page 92 and 94). Kabalka then describes that the liposomes are incorporated by RES cells (page 89); thus, in the Examiner's opinion, in view of the Britannica article, Kabalka would inherently meet the claim limitations.

Applicants disagree and point out that Kabalka is totally silent on any possible degradation of the liposomes by enzymes or effectors inside RES cells, let alone on degradation resulting in the formation of a water-soluble positive MR-imaging probe; actually, Kabalka's liposomes are so insoluble that they remain in the liver indefinitely, especially those which contain Gd-DTPA-SA (page 94, second paragraph).

Thus, Kabalka describes MRI contrast agents that act as such, *i.e.* without requiring degradation of the insoluble liposomes by enzymes or effectors in the surrounding environment so as to form water-soluble positive MR-imaging probes, allowing to record MRI images.

Second, Kabalka fails to disclose a method of cellular labeling. Instead, Kabalka merely describes paramagnetic liposomal agents for use as MRI contrast agents, especially for the imaging of the liver (see, for instance, the abstract), without disclosing or even suggesting their use in a method of cellular labeling. Thus, Kabalka fails to disclose a method of cellular labeling.

Considering this, Kabalka and the Britannica article do not meet all the limitations of the present claims and therefore they do not anticipate the subject matter of the present invention.

For the above reasons, none of the cited references (Klaveness, Shitaka or Kabalka) anticipate independent claim 1. For the same reasons the cited references do not respectively anticipate claims 2-3 and 6; 4, 6 and 8-10; or 2, 4-7 and 12, each of which depend directly or indirectly from claim 1. Applicants therefore respectfully request withdrawal of the § 102 rejections.

35 U.S.C. § 103

Claims 1, 2 and 4-13 were rejected as obvious over the combination of Klaveness and U.S. Pat. No. 5,498,421 ("Grinstaff") because, *inter alia*, Klaveness teaches degradable water-insoluble macromolecular particles and Grinstaff teaches a polymeric shell formulated from a biocompatible material.

In order to establish obviousness, it is necessary, *inter alia*, to (i) determine the scope of the prior art and (ii) the differences between the claimed subject matter and that of the prior art. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). Furthermore, a *prima facie* finding of obviousness cannot be established when the "improvement is more than the predictable use of prior art elements according to their established functions." *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct 1727, 1739 (2007). A reasonable expectation of success is required. MPEP 2143.02. Unexpected, *i.e.* surprising, results rebut a *prima facie* case of obviousness. MPEP 2144.09.

Applicants respectfully traverse the § 103(a) rejection because, for the reasons discussed below, (i) the cited combination fails to teach or suggest all of the claim limitations, (ii) the improvement was not predictable and (iii) there are surprising and unexpected results.

The Cited Combination Fails to Teach or Suggest All of the Claim Limitations

None of the cited references, alone or in combination, teach or suggest a method of cellular labeling wherein degradation of insoluble microparticles internalized into a targeted cell is necessary to form MR-imaging probes that would allow the registering of MRI images by T1–weighted sequences. Furthermore, none of the cited references, alone or in combination, teach or suggest exploiting a specific enzymatic activity in a targeted cell in order to activate insoluble particles to a pool of water-soluble contrast agent units and to record MRI images reflecting the enzymatic expression and activity. Only if the administered insoluble particles are degraded by enzymes or effectors inside the cell they can form positive MRI-imaging probes, i.e. free complex units (page 1, line 16-17) which provide positive contrast enhancement (page 3, line 24) or which promote a progressive reduction of the water-proton longitudinal relaxation time (page 5, lines 10-13), thus allowing recording of MRI images of the concerned cells by use of T1-weighted sequences.

In other words, degradation of the "relaxometrically silent" insoluble particles, to "relaxometrically active" species (page 4, lines 5-6), i.e. water-soluble MR-imaging probes, is necessary for their activation and for the obtainment of active MRI-imaging probes that allow to record MRI images by use of T1 -weighted sequences. This degradation/activation is promoted not only by naturally occurring enzymes (page 5, line10), but also by suitable enzymes expressed by molecular biology techniques (page 5, lines 16-17).

Since the amount of the released soluble complex depends on the amount of the enzyme destroying the particle, the method of the invention provides a contrast that

acts as a reporter of the enzymatic activity in the cells (from page 6, line 27 to page 7, line 4). Thus, in a further aspect, the method of the invention allows to register MRI images reflecting the local expression of the said specific enzyme.

In contrast, Klaveness merely describes degradable water-insoluble macromolecular particles which, after degradation, are taken up by the recticuloendothelial system (RES), thus allowing imaging of the liver. Klaveness neither teaches nor suggests the possible use of the macromolecular particles for cellular imaging, thus it fails to suggest a method of cellular labeling, let alone a method of cellular labeling wherein macromolecular particles are used to provide images reflecting the enzymatic activity of certain enzymes inside the cells.

Grinstaff fails to cure the deficiencies of Klaveness. Instead, Grinstaff merely describes compositions for in vivo delivery of biological compounds in the form of microparticles that are suitable for parenteral administration in aqueous suspensions (column 6, lines 9-12). Importantly, injectable suspensions are said to exhibit "organ targeting specificity (e.g. liver, spleen, lung, and the like) due to uptake of the polymeric shells of the invention by the RES or MNP system" (column 7, lines 18-21). Grinstaff is totally silent on the possible use of the described compositions for the delivery of a biological compound into a targeted cell.

The Improvement Was Not Predictable

Even if combining Klaveness with Grinstaff would lead to the claimed invention, which applicants maintain it would not, such an improvement would not have been predictable.

More specifically, the improvement is not predictable because, as described above, both Klaveness and Grinstaff refer to uptake by organs not cells. Since Grinstaff fails to mention or suggest a system for the delivery of a biological compound including insoluble particles to a cell, it would not have been predictable to provide a method of cellular labeling requiring internalizing an insoluble particle into a cell by combining the organ targeting in vivo delivery systems of Grinstaff with Klaveness' water-insoluble macromolecular particles, which are not targeted to cells. Nor would it have been predictable to degrade an internalized water insoluble particle by local enzymes to provide water soluble MRI-imaging probes. For the same reasons, there would have been no reasonable expectation of success in making the claimed invention.

Thus a person skilled in the art facing the problem of providing a method of cellular labeling and having knowledge of Grinstaff and/or Klaveness, would not have predicted the use of insoluble particles internalized in targeted cells and then degraded by enzymes or effectors inside the cells in order to be able to provide positive MRI-imaging probes.

Surprising Unexpected Results

As explained above, the method of the present invention allows one to register MRI images of cells, said images reflecting the expression of a specific enzyme in those cells. The ability to reflect the expression of a specific enzyme in cells is indeed surprising and it could not have been envisaged from the teaching of any one of the cited references, either alone or in combination with one another, since they do not contain any hint or suggestion to register MRI images of certain cells in order to measure enzymatic expression and activity.

Therefore, for the above reasons applicants respectfully submit that claim 1 is not rendered obvious by the combination of the cited references. For the same reasons claims 2 and 4-13, which depend directly or indirectly from claim 1, are not obvious over the cited combination. For these reasons, applicants request withdrawal of the § 103 rejection.

Conclusion

Therefore, for the above reasons, applicants submit that the presently pending claims are in condition for allowance and request the speedy issuance of a notice of allowability.

No fee, except the fee for a one month extension of time, is believed to be due for the filing of this Amendment and Response to Final Office Action. However, the Director is hereby authorized to charge any required fees and credit any overpayments to Deposit Account No. 50-0540.

Respectfully submitted,

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